

3. The method of claim 1, further comprising step (d) selecting recombinant polynucleotide sequences formed at step (c) that exhibit advantageous characteristics compared to corresponding characteristics of one or several reference sequences.

4. The method of claim 3, wherein the bank of polynucleotide sequences contains double-stranded polynucleotide sequences and step (a) further comprises denaturing the fragments obtained at step (a).

5. The method of claim 1, wherein the bank of polynucleotide sequences contains single-stranded polynucleotide sequences.

6. The method of claim 1, wherein at least one assembly matrix is double-stranded and it is first denatured and then added in single-stranded form at step (b).

7. The method of claim 1, wherein at least one assembly matrix is single-stranded.

8. The method of claim 1, further comprising at the end of step (c) at least one repetition of steps (a), (b), or (c).

9. The method of claim 1, further comprising at the end of step (c) at least one repetition of steps (b) or (c).

10. The method of claim 3, further comprising at the end of step (d), a step of choosing at least one recombinant polynucleotide sequence formed at step (c) and using the chosen sequence as a source of fragments or as an assembly matrix during at least one repetition of steps (a), (b), (c), or (d).

11. The method of claim 3, further comprising, before step (d), separating recombinant polynucleotide sequences formed at step (c) from the assembly matrix.

12. The method of claim 3, further comprising, before step (d), amplifying recombinant polynucleotide sequences formed at step (c).

13. The method of claim 3, further comprising before step (d), cloning the recombinant polynucleotide sequences formed at step (c).

14. The method of claim 1, wherein the ends of the fragments provided at step (a) are such that the fragments hybridize adjacently to one another on the assembly matrix.

15. The method of claim 14, wherein steps (b) and (c) are performed simultaneously.

16. The method of claim 14, wherein step (a) comprises subjecting the polynucleotide sequences of the initial bank to hydrolysis by the action of one or several restriction enzymes.

17. The method of claim 14, wherein step (a) further comprises randomly fragmenting polynucleotide sequences from the bank of polynucleotide sequences.

18. The method of claim 1, further comprising adding an enzyme at step (b) or (c) that specifically recognizes and degrades any nonhybridized ends of the fragments when said nonhybridized ends cover other hybridized fragments on the same matrix.

19. The method of claim 18, wherein the enzyme is Flap endonuclease.

20. The method of claim 18, wherein the enzyme is a single-stranded exonuclease.

21. The method of claim 1, wherein a thermostable ligase that is active at high temperature is used at step (c).

22. The method of claim 18 or 19, further comprising using a thermostable ligase at step (c), wherein the enzyme that recognizes and degrades the nonhybridized ends is as thermostable as the thermostable ligase.

23. The method of claim 20, further comprising using a thermostable ligase at step (c), wherein the enzyme that recognizes and degrades the nonhybridized ends is as thermostable as the thermostable ligase.

24. The method of claim 1, wherein the initial bank of polynucleotide sequences is produced from a wild gene by successive steps of controlled mutagenesis, by error prone PCR, by random chemical mutagenesis, by *in vivo* random mutagenesis, or by combining genes of near or distinct families within the same species or from different species so as to make available a variety of polynucleotide sequences in the initial bank.

25. The method of claim 1, wherein the initial bank of polynucleotide sequences consists of synthetic sequences.

A4  
cost.

26. The method of claim 1, wherein step (a) comprises subjecting the initial bank to hydrolysis by the action of several different restriction enzymes or by the action of restriction enzymes having a large number of cutting sites on the polynucleotide sequences of the initial bank.

27. The method of claim 17, wherein randomly fragmenting polynucleotide sequences from the bank of polynucleotide sequences comprises treating the polynucleotide sequences with DNAase I.

28. The method of claim 17, wherein a fragment produced by the random fragmenting is used as the assembly matrix at step (b).

29. The method of claim 26, wherein a fragment obtained at step (a) by a treatment with restriction enzymes is used as the assembly matrix at step (b).

30. The method of claim 1, wherein the fragments of step (a) are obtained by amplification reactions performed on polynucleotide sequences of the initial bank using initiated oligonucleotides, making it possible to produce fragments having sequences in

common, said fragments acting as an assembly matrix for one another at step (b) or at step (c).

31. The method of claim 2, wherein the polynucleotide sequences of the initial bank are fragmented into three or more fragments.

32. The method of claim 1, wherein, in addition to the fragments and assembly matrix, oligonucleotides of varying length, single- or double-stranded, are added at step (a) or (b).

A4  
cost. 33. The method of claim 3, wherein, before step (d), the recombinant polynucleotide sequences formed at step (c) are separated from the assembly matrix thanks to a marker present on the assembly matrix or on the recombinant polynucleotide sequences.

34. The method of claim 3, wherein the recombinant polynucleotide sequences formed at step (c) are used to select the recombinant polynucleotide sequences, formed during subsequent repetitions of step (c), that have advantageous characteristics compared with corresponding characteristics of reference sequences.

35. The method of claim 34, wherein the screening is performed by *in vitro* expression of recombinant polynucleotide sequences.

36. The method of claim 1, wherein the initial bank of polynucleotide sequences comprises one or several restricted banks prepared by a prior performance of the method of claim 1.

---

Please add the following new dependent claims.

A5 42. The method of claim 1, wherein the fragments are random fragments.

43. The method of claim 2, wherein the fragmenting is random.
44. The method of claim 1, 2, 3, 42 or 43 wherein the method is performed *in vitro*.

45. The method of claim 44, wherein the fragments hybridize adjacently to one another on the assembly matrix and require no polymerization to ligate to each other.

AS  
CDU+. 46. The method of claim 45, wherein step (c) is performed without a polymerase.

47. The method of claim 46, further comprising, after step (c), amplifying the recombinant polynucleotide sequences formed at step (c) with PCR amplification.

48. The method of claim 45, wherein the method is performed without a polymerase.

49. The method of claim 1, further comprising separating the recombinant polynucleotide sequences formed at step (c) from the assembly matrix and, before step (d), cloning the recombinant polynucleotide sequences. --

---

#### REMARKS

Applicants respectfully request entry of the above amendments to pending claims 1-36. Applicants also request entry of new dependent claims 42-49.<sup>1</sup> Claims 37-

---

<sup>1</sup> Please note that new claims 45, 46 and 48 contain the phrase "no polymerase" or "without a polymerase." Applicants appreciate that the PTO tends to prefer positive limitations. However, negative limitations are clearly allowed if they have literal or implicit support in the original disclosure. See MPEP 2173.05(i). Support can be found, for instance, on page 2 of the application, first and second paragraphs.